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Effects of temperature, pre-chilling and light on seed germination of *Withania somnifera*; a high value medicinal plant

L. Kambizi [a], P.O. Adebola [b], A.J. Afolayan [a,*]

[a] *Botany Department, University of Fort Hare, Alice 5700, South Africa*
[b] *Plant Breeding Group, Cocoa Research Institute of Nigeria, PMB 5244, Ibadan, Nigeria*

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Abstract

Withania somnifera is under intensive utilization because of its wide ranging medicinal potential. Self-propagation of this species is by seed. In an effort to improve and promote the cultivation of this over-exploited medicinal herb, the effects of temperature and light on the germination of its seeds were investigated. Germination was found to be temperature and light-dependent. Although seed viability was 78.8% as determined by tetrazolium staining, maximum germination was only 46%. Pre-chilling treatments reduced the germination of the seeds, while exposure to constant temperatures of 25 °C, 35 °C and 45 °C in the dark completely inhibited germination. A regime of alternating temperatures partially suppressed the light requirement and improved germination of the seeds.

Keywords: Germination; Medicinal plant; Pre-chilling; Temperature; *Withania somnifera*

1. Introduction

Withania somnifera (Solanaceae) is a plant of repute in the Indian and African systems of traditional medicine. It is generally useful as an abortifacient, amoebicide, bactericide and contraceptive (Asthana and Raina, 1989; Devi, 1996). The plant is used as anti-stress, anti-inflammatory, anti-arthritic, anti-oxidant and anti-tumor agent (Uma-Devi et al., 1992, 1993; Singh and Kumar, 1998). These properties were attributed to the presence of a number of alkaloids in the roots and steroidal lactones in the leaves (Kirson et al., 1971; Scartezzini and Speroni, 2000). The plant is an erect, perennial shrublet with densely velvety stems and leaves. It grows to a height of 1.5 m with a spread of 1 m. It occurs mainly in KwaZulu-Natal, Free State and the Eastern Cape provinces of South Africa where the local inhabitants have been using it since time immemorial as a remedy for a number of diseases. Extracts from this herb have been used for centuries by some indigenous people of South Africa against sexually transmitted infections, asthma and as an anti-inflammatory agent (Van Wyk et al., 1997). Methanol and acetone extracts from its roots have shown significant activities against Gram-positive and Gram-negative bacteria (Afolayan et al., 2002).

In our previous ethnomedical study (Kambizi and Afolayan, 2003), we observed that the populations of this plant have become low and scattered in the wild, which may be attributed to its intensive harvesting for tradomedicine coupled with its low potential for natural regeneration. Self-propagation of the plant is by seed (Kattimani and Reddy, 1999) and the germination potential of the seeds has been reported to be very low (Vakeswaran and Krishnasamy, 2003b). Propagation by natural re-seeding is thus no longer sufficient to guarantee the survival of this plant. It has become necessary, therefore, to develop rapid methods of cultivating the plant in order to ensure its sustainable utilization in the Eastern Cape. According to Kothari et al. (2003), the plant has a high potential for commercial cultivation. Before this goal could be achieved, however, basic information on the best local conditions for the germination of its seeds is very essential. Previous study on the germination of the seeds of *W. somnifera* was focused on the influence of growth regulators. The study revealed that

* Corresponding author.
E-mail address: aafolayan@ufh.ac.za (A.J. Afolayan).

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gibberellic acid improved seed germination in this plant when compared to other growth regulators (Vakeswaran and Krishnasamy, 2003b). In this study, we report on the effects of temperature and light on the germination of its seeds.

2. Materials and methods

2.1. Seed collection

The fruits of *W. somnifera* used for this experiment were harvested from its natural populations within the Nkonkobe Municipality of the Eastern Cape Province (latitudes 30°00′–34°15′S and longitudes 22°45′–30°15′E). The seeds were removed from the berries, air dried and stored in paper bags at room temperature (15–25 °C). The various trials were conducted 3 weeks after collection. Only mature seeds were used in the experiments.

2.2. Viability test

To ensure that the seeds used for the experiment were viable and of high quality, the sample lot was subjected to viability test using the tetrazolium technique (Grabe, 1970). Using the procedure for the Solanaceae (Peters, 2000), the seeds were imbibed for 24 h in water, cut along the margin without damaging the embryo and soaked in colourless 0.1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) solution for 16 h at 25 °C in the dark. The seeds were then removed from TTC solution, washed with distilled water and soaked in 10 ml of 95% ethyl alcohol to permit direct observation of the embryo. The embryos of viable seeds appeared reddish in colour. Three replicates of 50 seeds each were examined.

2.3. Germination tests

Germination trials were conducted in 9-cm sterile Petri dishes lined with two Whatman No. 1 filter papers and moistened with sterile distilled water to ensure adequate moisture for the seeds. Treatments were arranged in a factorial experiment (randomized complete block) with three replicates of 50 seeds each. Seed treatments included continuous light, continuous darkness and 16/8 h light/dark photoperiod. Other treatments were: 1, 3 and 7 days pre-chilling at 4 °C. The effect of temperature was determined by placing the Petri dishes in a germination chamber (incubator) for 43 days at constant temperatures of 25 °C, 35 °C, 45 ° and under alternating day/night temperature (18–25 °C) and light (16 h daylight/8 h night) as outlined by the Association of Official Seed Analyst (AOSA, 1993). In an attempt to remove germination inhibitors, the seeds were leached with distilled water for 5 days before the experiment. Seeds were then surface sterilized in aqueous solution of 0.1% mercuric chloride for 60 s to prevent fungal attack and rinsed in several changes of sterile water. For treatment subjected to continuous darkness, the Petri dishes were covered with aluminium foil and daily observations were carried out under green safe light. Light treatments were exposed to continuous illumination produced by white fluorescent tubes with a mean proton flux density of 24 μmol^{-2} s^{-1}. The fluorescent tubes also provided the source of red light. The seeds were examined daily and considered germinated when the radicle was visible. At the end of the trials, data were subjected to analysis of variance procedures and mean separation using SAS statistical packages.

Table 1
Analysis of variance of seed germination in *Withania somnifera* (Data in randomized complete block design and 3 × 3 factorial)

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F[b]	Tabular F 5%	1%
Replication	2	4.38	2.19	1.41[ns]	3.44	5.72
Treatment	11	1349.63	122.69	79.15[s]	2.26	3.18
Light regimes (A)	(2)	1134.88	567.44	366.09[s]	3.44	5.72
Pre-chilling days (B)	(3)	177.41	59.13	38.14[s]	3.05	4.82
A × B	(6)	37.41	6.23	4.02[s]	2.55	3.76
Error	22	34.28	1.55			
Total	35	1388.30				

ns = not significant, s = significant at (P > 0.01).

Table 2
Germination of *Withania somnifera* seeds at three different pre-chilling conditions and three photoperiodic regimes

Pre-chilling treatment	Germination %		
	Continuous light	Continuous darkness	Alternate light/dark (16/8h photoperiod)
Untreated (Control)	18.7 (0.4)[a]	35.3 (0.47)[b]	46.0 (0.81)[a]
One day	17.3 (1.41)[a]	34.0 (1.69)[b]	44.7 (0.01)[a]
Three days	16.0 (0.81)[a]	38.0 (0.94)[a]	34.0 (1.69)[b]
Seven days	10.0 (0.81)[b]	18.0 (0.47)[c]	36.0 (1.24)[bc]

Note: Each value represents mean percentage germination with standard deviation in parenthesis. Means within the same column followed by the same superscript do not differ significantly (P > 0.01). At constant temperature the seed did not germinate, hence the data are not shown.

3. Results and discussion

Tetrazolium chloride test have been shown to be generally in close agreement with germination test results and have been listed for over 650 plant species (Moore, 1985; Leist and Krämer, 2003). The viability of the seeds of *W. somnifera* as determined by the tetrazolium test was 78.8%. This high percentage viability is probably due to the short period between the harvesting and the trials of the seeds (3 weeks). This is because viability in the seeds of this plant is reported to decrease with time. According to Vakeswaran and Krishnasamy (2003a), seed viability in *W. somnifera* can be as low as 5% at the end of 1 year. To obtain maximum germination results, current season seeds are therefore recommended for planting.

The result of this study clearly showed that temperature and photoperiod affected the germination of *W. somnifera* seeds. The influence of these two conditions is significant when considered in isolation or when the interaction between them is taken into account (Table 1). The optimum condition for germination of the seeds in this experiment was alternating temperatures of 18–25 °C under 16/8 h photoperiod (Table 2). Germination under light and dark was significantly higher than under continuous light or continuous darkness. Similar observations were recorded by Schonbeck and Egley (1980) on redroot pigweed seeds (*Amaranthus retroflexus*). This type of light-controlled germination has been associated with phytochrome since the pioneer work of Borthwick (1952). The sensitivity of seeds to the spectral quality of the light mediated by phytochrome is a frequent natural process within species that grow in open areas (Ballaré, 1994). According to Godoi and Takaki (2004), the requirement of alternating temperatures to optimize seed germination is a common behaviour and has been observed in many plant species. Ellis and Barret (1994) suggested that this mechanism prevents germination when cool daytime temperatures are followed by possible very cold nights. This necessity, most probably, reflects an adaptation to natural fluctuation of the habitat or may be associated with dormancy process, which most often confers an adaptive advantage on the species.

W. somnifera seeds did not germinate when exposed to constant temperatures in continuous darkness. On the other hand, germination was recorded (35.3%) under a regime of alternating temperatures in continuous darkness. Light is one of the most important environmental factors that interact with temperature to regulate seed germination in many plant species (Baskin and Baskin, 1998), but light requirement for germination may vary with temperature (El-Keblawy and Al-Rawai, 2005). This result implies that the seeds of *W. somnifera* are light-dependent for germination but exposure to regimes of alternating temperatures may probably suppress the effect of the photoperiod. In a similar work, Zaia and Takaki (1998) observed that the seeds of *Tibouchina pulchra* and *Tibouchina granulose* did not germinate in darkness at constant temperatures, while 40% germination was recorded under a regime of alternating temperatures in the dark. Fluctuating temperatures on a daily or seasonal basis are major requirements for seed germination in some plants. In addition, temperature has been reported to be interrelated with light for seed germination in certain plants (Relf, 1997) and, according to Hartmann and Kester (2001), alternating day and night temperatures for some plant species give better germination results than constant temperatures. The daily fluctuation in soil temperature may therefore have a significant influence on the germination response in *W. somnifera*.

Subjecting the seeds to low temperatures (4 °C) prior to germination resulted in a significant negative effect on the percentage germination when compared to the control (Table 2). Although cold stratification, has been reported to break dormancy of viable seeds and enhance germination in many species (Baskin et al., 2001), it has also been reported to cause lethal effect on viable seeds (Ren and Tao, 2004). The latter might have been the case with *W. somnifera* seeds when they were subjected to pre-chilling treatment in this experiment.

The maximum percentage germination (46%) obtained in this experiment is still very low, considering the fact that TTC test indicated 78.8% seed viability. This discrepancy explains why there is low natural regeneration with consequent low plant populations of *W. somnifera* in the wild. From this study, it is evident that germination of seeds of this plant in the wild would be largely dependent on light and temperature conditions. These conditions and hormonal additives (gibberellins) should be taken into consideration in the economic cultivation of this valuable plant.

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